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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Timothy J. O'Brien Examiner: F.B. Hoffer

Serial No.: 042,498 Art Unit: 182

Filed: April 24, 1987 Docket: 6626

For: TUMOR SPECIFIC ASSAY FOR CA125 OVARIAN CANCER ANTIGEN

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. 1.132

I, TIMOTHY J. O'BRIEN, declare that:

1. I am the sole inventor of the subject matter of the above-identified patent application; I am familiar with the contents of said application and have read the Office Action dated December 20, 1988, and the Advisory Action dated May 19, 1989 issued in connection therewith.

2. I received an undergraduate degree in Biochemistry from University College, Dublin, Ireland, in 1961; an M.S. in Biology from University of Toronto, Toronto, Canada, in 1964; and a Ph.D. in Biochemistry from University of Illinois, Champagne, Illinois, in 1967. I was employed as a postdoctoral fellow at Purdue University, West Lafayette, Indiana, from 1967-1969; as a Helen Hancock Research Associate at University of Southern California, Los Angeles, California, from 1969-1974; as a postdoctoral fellow at City of Hope National Medical Center, Duarte, California, from 1974-1978; as an Assistant Professor from 1978-1984 and an Associate Professor in 1984 at University of Southern California; and since 1984 as an Associate Professor at University of Arkansas for Medical Sciences, Little Rock, Arkansas.

3. I am a citizen of the United States residing at 2625 Gristmill Road, Little Rock, the State of Arkansas.

4. I caused to be carried out at my direction and under my

supervision certain experimental work which demonstrates that the invention embraced by the above-identified application directed to an antibody against a 40 kd subunit of the CA125 antigen would be unique with respect to CA125 recognition because the OC125 antibody (also known as the Bast antibody) only recognizes the 200 kd subunit of the CA125 molecule. It does not cross react with the 40 kd subunit.

The CA125 antigen was purified from ascites fluid obtained from a patient with ovarian cancer to high specific activity (ca. 2000-3000 units/ug protein). The purification scheme for the CA125 antigen was as described in the subject application at Page 17, Line 1 to Page 18, Line 3. SDS polyacrylamide gel electrophoresis of this highly purified antigen on a gradient polyacrylamide gel followed by silver staining indicated that the purified CA125 antigen is composed of several subunits. Among these subunits are proteins with apparent molecular weights of approximately 200 kDal, 116 kDal, 50 kDal, and 40 kDal (Fig. 1, attached hereto and made part hereof as Exhibit 1). The previously reported 92 kDal protein was not observed in this experiment. These proteins were electrophoretically transferred from a similar polyacrylamide gel to an Immobilon membrane filter for immunoblotting as described in O'Brien et al. (1986) Am. J. Obstet. Gynecol. 155:50-55. The filters were probed with ^{125}I-OC125 antibody and an autoradiograph of the blot is shown in Fig. 2 (attached hereto and made part hereof as Exhibit 2). The results indicate that the OC125 antibody recognized the 200 kDal subunit and, further, that the OC125 antibody does not bind to any of the lower molecular weight proteins described above, including and especially the 40 kDal protein. Therefore, the OC125 antibody does not cross react with the 40 kDal antigen of the present invention and must therefore be distinct from the subject antibodies which react with the 40 kDal antibody.

5. In my opinion, the data indicate that no cross reactivity exists

between the OC125 antibody which recognizes only the 200 kDal subunit of the CA125 antigen and an antibody which recognizes only the 40 kDal subunit thereof; and therefore, that the subject antibody and the OC125 antibody have different antigenic specificities and are distinct with respect to each other.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Oct 10/1989

Timothy J. O'Brien

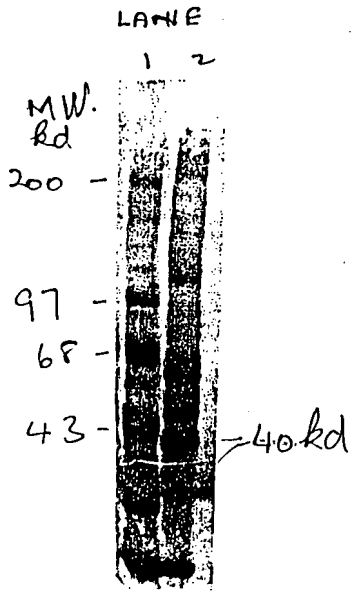

Figure 1

CA125 Subunits
Silver Stained

Figure 1. SDS polyacrylamide gel electrophoresis of high specific activity CA125 antigen. The gel was stained using the Biorad silver stain protocol. Lane 1 molecular weight markers as indicated. Lane 2 purified CA125.

Figure 2

Western Blot CA125
Using ^{125}I-OC125

Figure 2. Western blot of CA125 after SDS electrophoresis followed by transblotting to immobilon filter. Filter was treated with blotto to prevent non specific binding and probed with (5 mls of 10^6cm/ml) ^{128}I-OC125 as provided in the standard CA125 kit. The blot was exposed to x-ray film to detect the localized ^{125}I-OC125 binding.